

LABORATORY STUDIES ON THE CHEMISTRY OF SOFT CURD MILK

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LABORATORY STUDIES ON THE CHEMISTRY OF SOFT CURD MILK

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Much interest in soft curd milk has developed since Hill (1) showed how to differentiate the milks of individual cows with respect to curd character and indicated the potential value of soft curd milk for infant feeding. Hill (2) (3) has since devoted most of his attention to studies on the distribution of curd character in herds, environmental and hereditary factors, and to the clinical aspects of his findings.

Espe (4) (5) has made an excellent experimental study of the digestibility of milk in relation to curd character and has given some attention to the chemistry of soft curd milk.

Our work carried on independently of the above workers consists primarily of analytical and physico-chemical studies on the constitution of soft curd milk.

PART I. THE QUANTITATIVE COMPOSITION OF SOFT AND HARD CURD MILKS IN RELATION TO CURD CHARACTER

A fairly thorough study was made of the concentration of casein, fat, whey proteins, calcium, phosphorus, citric acid, and lactose in relation to curd character. These constituents will be dealt with both individually and, as far as possible, collectively, in this portion of the paper.

The curd character was determined by means of the Hill test. This test affords a fairly quantitative measure of the compactness of the milk curd upon peptic digestion. It is fully described by Hill (1) in one of his bulletins. Briefly, it consists of adding a standard reagent consisting of 3 parts of an 0.6 per cent pepsin solution and 1 part of calcium chloride solution (378 grams made up to one liter) to 100 cc. of milk brought to body temperature. The milk coagulates rapidly about a set of radial knives which are drawn up through the curd and resistance offered is read off on a spring balance in grams. The readings are most readily reproducible for milks that give a loose curd (soft curd milks) so that one may check on duplicate determinations exactly or within 5 grams. With milks that give a relatively stiff curd the accuracy of reproduction is lower, duplicate readings may vary approximately 10 grams. When the milk is extremely hard curded the variations may be considerably greater.

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A. Concentration of Casein

Casein determinations were made by the method of Waterman (7) on several soft and hard curd milks. The data obtained are presented in table 1.

TABLE 1
Concentration of casein and whey proteins in relation to curd character

COW NO.	CASEIN	WHEY PROTEINS	RATIO: $\frac{\text{CASEIN}}{\text{WHEY PROTEINS}}$		CURD TENSION
	<i>per cent</i>	<i>per cent</i>			<i>gms.</i>
32-51	1.85	0.64	2.9		28
10-5	2.10	0.64	3.3		28
20-5	2.17	0.51	4.2		30
4-5	2.17	0.61	3.6		58
32-4	2.24	0.89	2.5		22
19-5	2.36	0.61	3.9		32
13-5	2.49	0.64	3.9		30
26-5	2.66	0.86	3.1		58
28-5	2.91	0.72	4.0		40
38-5	3.07	0.70	4.4		75
45-5	3.07	1.05	2.9		110
32-52	3.19	0.78	4.1		120
14-4	3.32	0.90	3.7		85
37-5	3.51	0.76	4.6		140
31-4	3.71	0.92	4.0		163
36-4	4.25	0.98	4.3		190

It is evident from table 1 that on the average the concentration of casein in soft curd milk is definitely lower than in hard curd milk. This same conclusion has been independently arrived at by Hill (8) and by Espe (4). However, inspection of table 1 shows that the correlation between curd tension and concentration of casein is by no means ideal.

Espe states (4) that the variation in the percentage of casein accounts for about 81 per cent of the difference in curd tension between hard and soft curd milks. We do not feel it possible to assign so definite a figure. Nevertheless, it may be said that the low percentage of casein is a very important factor in determining the curd character of soft curd milk but that other factors also operate.

B. Whey Protein Concentration

The percentage of whey protein was determined from the nitrogen content of the filtrate obtained after precipitating the casein by the method of Waterman. In table 1 are presented the results of these determinations as well as the ratio of the casein to the whey protein content.

Some indication may be seen of a slightly lower whey protein content in the soft curd milk but it is not as clearly defined as for the casein. There

is some indication also that ratio of casein to whey proteins is generally lower for the soft curd milk.

A high ratio of whey proteins to casein might conceivably be the basis of soft curd milk. In order to test this idea a milk was analyzed for its casein and whey protein content and then to it were added progressive amounts of whey protein powder obtained from the Division of Dairy Industry, Department of Agriculture. The curd tension was determined for each concentration of whey proteins, the concentration of casein remaining constant. The data obtained are presented in table 3. Series A represents a hard curd milk, Series B, a soft curd milk.

TABLE 2
Effect of increasing concentration of whey proteins upon the curd tension

SERIES A	CASEIN	WHEY PROTEINS	RATIO : $\frac{\text{CASEIN}}{\text{WHEY PROTEINS}}$		CURD TENSION
	<i>per cent</i>	<i>per cent</i>			<i>gms.</i>
Cow No. 335	3.39	0.76	4.5		132
	3.39	0.96	3.5		142
	3.39	1.16	2.9		135
	3.39	1.36	2.5		140
	3.39	1.55	2.1		132
	3.39	1.75	1.9		128
SERIES B	2.27	0.51	4.5		60
8 parts	2.27	0.71	3.2		58
No. 335	2.27	0.91	2.5		60
with 4 parts	2.27	1.11	2.1		60
water	2.27	1.30	1.8		57
	2.27	1.50	1.5		50

The data of table 2 indicate that the ratio of casein to whey proteins can play but a small part, if any, in determining the curd character. Moreover, the data would seem to show that the concentration of whey proteins alone plays a negligible part in determining the curd character.

C. Concentration of Fat in Relation to Curd Character

In table 3 are presented the results of several fat determinations on milks of known curd tension.

It will be seen from table 3 that the average percentage of fat in soft curd milk tends to be lower than in hard curd milk but individual variations are large—for example, one soft milk in the table has 5.6 per cent fat and two hard curd milks 3.8 per cent fat.

Hill (2) presented data which show that the removal of the fat from milk causes an increase in curd tension but that the concentration of fat in a milk is not an index to the curd character.

We believe that no simple correlation between fat content and curd character is to be expected since the distribution of the milk fat in relation to the other milk constituents is probably at least as significant as its concentration. That the manner in which the fat is dispersed varies considerably from one individual milk to another seems to be indicated by our work and work done at the New York Agricultural Experiment Station at Geneva.

Experiments which we have made relating homogenization pressure to curd tension clearly show that increasing the dispersion of the fat of a

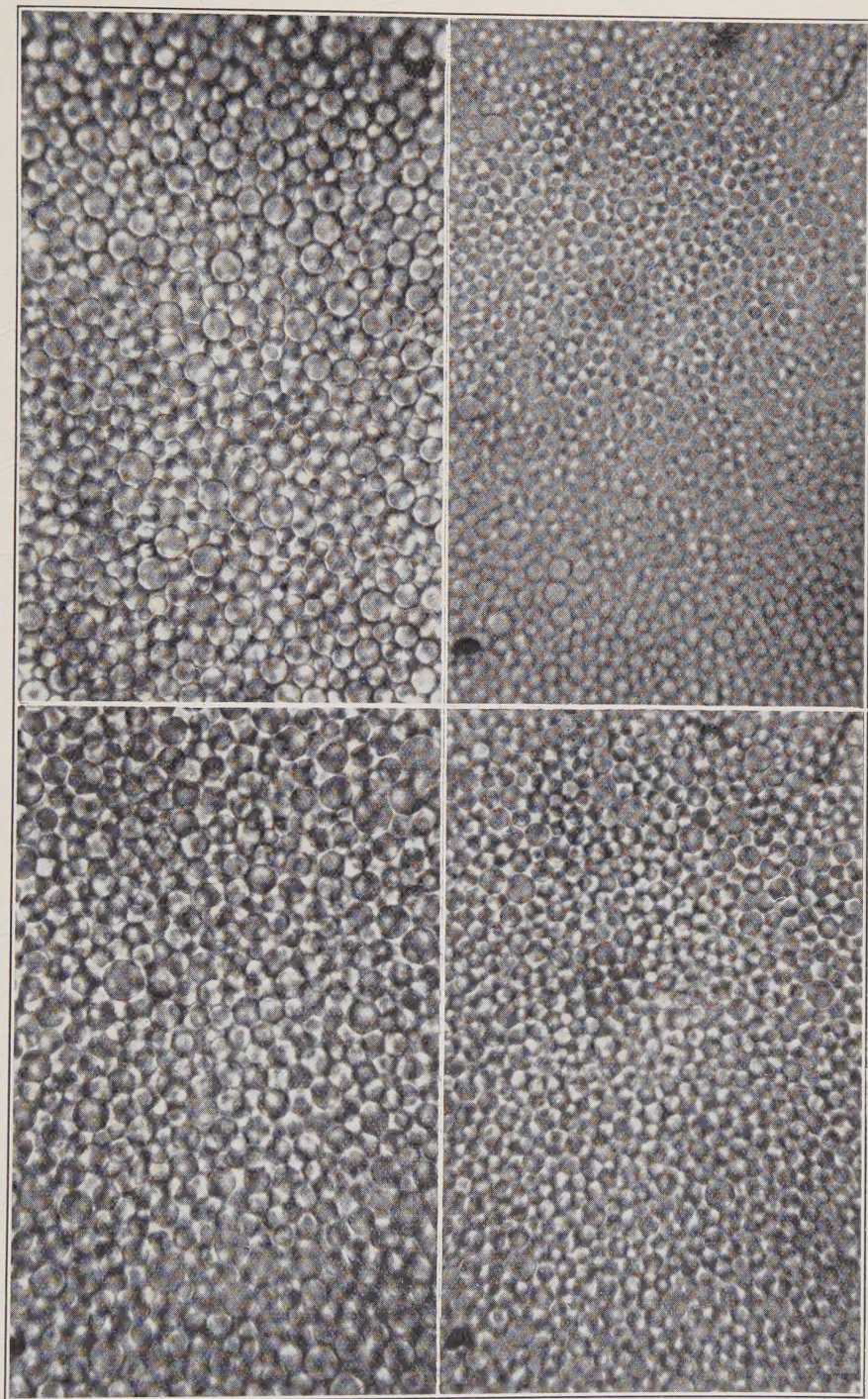
TABLE 3
Concentration of fat in relation to curd character

FAT	CURD TENSION	FAT	CURD TENSION
<i>per cent</i>	<i>grams</i>	<i>per cent</i>	<i>grams</i>
3.3	32	6.8	110
3.2	39	5.9	65
3.4	40	7.8	85
5.0	30	5.4	75
4.3	30	6.8	70
4.0	32	3.8	60
5.4	30	7.8	55
3.6	25	5.8	125
3.6	40	3.8	102
4.5	45	4.2	102
4.3	38		
4.2	28		
5.6	28		
4.2	30		
Average 4.2		Average 5.8	

milk causes a considerable lowering in the curd tension compared with that of the original milk.

Hill's data indicate that the removal of cream may increase the curd tension as much as 20 per cent. The concentration and distribution of the fat unquestionably play an important part in determining the curd character but it is an involved problem to quantitatively evaluate the two effects and we have not attempted its solution at this time.

It seemed a reasonable supposition that the size of the fat globules of the individual milk might bear some relation to the curd character. To test this concept photomicrographs of the fat globules of individual milks were made using a technique similar to that developed by Turner and Haskell (9). No correlation was found between curd character and size of fat globules as may be seen from the photomicrographs.



Size of fat globules in hard and soft curd milks— $\times 744$: No. 2, hard curd Guernsey milk (upper left); No. 285, soft curd Guernsey milk (upper right); No. 345, hard curd Holstein milk (lower left); No. 225, hard curd Holstein milk (lower right). Scale, 0.01 mm. = 0.3 inches

D. Concentration of Lactose

In mixed soft curd milk we found the lactose concentration to be 4.8 per cent and in mixed regular certified milk we found 4.7 per cent lactose. Hill (8) found 4.32 per cent in soft curd Holstein milk and 4.68 per cent in hard curd Holstein milk.

In table 4 is shown the effect on the curd tension of adding increasing amounts of pure finely divided lactose.

TABLE 4
Effect of increasing concentration of lactose on the curd character

AMT. LACTOSE ADDED PER 100 CC. RAW CERTIFIED MILK	CURD TENSION
<i>gms.</i>	<i>gms.</i>
0.0	58
1.0	55
2.0	55
3.0	60
4.0	45
5.0	50
6.0	50

It will be seen from table 4 that the concentration of lactose appears to have little or no effect in influencing the curd tension, unless we assume, what is very improbable, that up to 4 or 5 per cent concentration the lactose does exert an effect and then beyond this concentration no longer does.

E. Calcium and Phosphorus in Relation to Curd Character

Calcium determinations were made according to a method given by Scott (10) and phosphorus determined by a method given in the same book. The data obtained are presented in table 5.

TABLE 5
Concentration of calcium and phosphorus in relation to curd character

DESCRIPTION OF MILK	Ca	P ₂ O ₅
	<i>per cent</i>	<i>per cent</i>
Mixed soft curd	0.12	
32-5 soft curd	0.09	0.23
7-5 soft curd	0.11	
10-5 soft curd	0.11	0.24
Mixed hard curd	0.15	
38-5 hard curd	0.15	0.34
3 hard curd	0.16	0.29

It will be noted that both the calcium and phosphorus are present in lower concentration in the soft curd milk but the ratio of calcium to phosphorus is substantially the same for the two types of milk.

F. *Concentration of Citric Acid in Relation to Curd Character*

According to Sherwood and Hammer (11) the citric acid content of individual milks examined by them varied from 0.07 to 0.33 per cent and the average citric acid content for a given cow's milk seemed to be fairly constant. These facts seemed significant and analyses were made using essentially the same method as that of the above authors.

In table 6 are presented the results of the determinations.

TABLE 6
Concentration of citric acid in relation to curd character

COW NO.	MEAN CURD TENSION	CITRIC ACID
	<i>gms.</i>	<i>per cent (by volume)</i>
105	32	0.23
195		0.28
425		0.24
		mean 0.25
335	130	0.28
375		0.31
415		0.24
		mean 0.28

The data in table 6 indicate no significant difference in the concentration of citric acid between soft and hard curd.

TABLE 7
Ash concentration in relation to curd character

COW NO.	CURD TENSION	ASH
	<i>gms.</i>	<i>per cent</i>
425	32	0.71
275	39	0.68
415	30	0.68
		mean 0.69
395	102	0.74
445	102	0.69
184	125	0.80
		mean 0.74

G. Ash Content in Relation to Curd Character

The amount of ash left after ashing down a sample of milk is an index to the original salt concentration. Table 7 contains some determinations made on hard and soft curd milks.

These data are not comprehensive enough to warrant any definite conclusion. However, according to some data of Hill (8) Holstein soft curd milk contained 0.63 per cent ash and Holstein hard curd milk, 0.74 per cent ash. Espe's data (4) indicate an average of 0.57 per cent ash for soft curd milk and 0.80 per cent for hard curd milk.

H. Whey Dilution Experiments

It was found possible by centrifuging the whole milk in a Sharpless super-centrifuge at 40,000 r.p.m. for 20 minutes to separate the "suspensoid" phase of the milk (the calcium caseinate, colloidal salts, fat) and from the bowl of the centrifuge was obtained a yellowish, transparent whey. From this fluid 93 per cent of the casein was found to be absent.

It was then possible to carry out whey dilution experiments with this liquor by diluting whole milk with it and making curd tension determinations.

In table 8 data are presented to show the effect of diluting a hard curd milk with its own whey and with whey from a soft curd milk.

TABLE 8
Whey interchange experiments

COW NO.	DESCRIPTION OF MILK	CURD TENSION
		<i>gms.</i>
4-4	Hard curd whole milk	122
4-4	70 cc. milk + 30 cc. distilled H ₂ O	
4-4	70 cc. milk + 30 cc. (4-4) whey	65
4-4	70 cc. milk + 30 cc. (28-4) whey soft curd	62
37-5	Hard curd whole milk	138
37-5	70 cc. milk + 30 cc. H ₂ O	90
37-5	70 cc. milk + 30 cc. (37-5) whey	75
37-5	70 cc. milk + 30 cc. (24-5) whey soft curd	88

Experiments of this type are naturally not ideal but it was hoped that a rough estimate of the importance of the whey constituents *as a whole* might be obtained in this way. Table 8 indicates that the soft curd whey seems to have the same influence on curd character as the hard curd whey.

To further test this matter, more extensive dilution of the milks was carried out. The data obtained are shown in table 9.

It is difficult to interpret the data of table 9 as the measurements of the Hill test have in general a precision of 5 to 10 grams and the variations fall

TABLE 9
Supplementary whey interchange experiments

DESCRIPTION OF MILK	CURD TENSION		DESCRIPTION OF MILK
	<i>gms.</i>		
28-4 soft curd whole milk	69.0	102.5	4-4 hard curd milk
50 cc. (28-4) milk			50 cc. milk (4-4)
+ 50 cc. H ₂ O	22.5	27.0	+ 50 cc. H ₂ O
50 cc. (28-4) milk			50 cc. milk (4-4)
+ 50 cc. (28-4) whey	12.5	25.0	+ 50 cc. (4-4) whey
50 cc. (28-4) milk			50 cc. milk (4-4)
+ 50 cc. (4-4) whey	17.5	20.0	+ 50 cc. (28-4) whey
24-5 soft curd whole milk	47.5	112.0	37-5 whole milk (hard curd)
50 cc. (24-5) milk	20.0	37.5	50 cc. (37-5) milk
+ 50 cc. H ₂ O			+ 50 cc. H ₂ O
50 cc. milk (24-5)			50 cc. (37-5) milk
+ 50 cc. 24-5 whey	10.0	30.0	+ 50 cc. (37-5) whey
50 cc. milk (24-5)			50 cc. (37-5) milk
+ 50 cc. 37-5 whey	15.0	22.5	+ 50 cc. (24-5) whey

within this limit. Possibly there is some indication here of a small specific whey influence. However, the dilutions employed in table 9 reduce the concentration of the casein to a point seldom encountered even with a soft curd milk.

SUMMARY OF PART I

1. The concentration of casein is shown to be lower in the soft curd milk than in hard curd milk. This fact is considered to account, to an important extent, for the lower curd tension of soft curd milk.

2. The soft curd milk contains less calcium and phosphorus than the hard curd milk.

3. The concentration of the individual whey constituents seems to exert little or no influence in determining the curd tension.

4. The whey constituents as a whole likewise seem to exert little if any influence in differentiating a soft curd milk from a hard curd milk.

PART II. PHYSICO-CHEMICAL MEASUREMENTS ON SOFT AND HARD CURD MILKS

A. Freezing Point and Conductivity Measurements

From measurements of the freezing points and conductivity one might hope to learn something of the "salt balance" of soft and hard curd milks. The characteristic freezing point of a milk is essentially due to the salt ions and lactose. The conductivity depends essentially on the nature and concentration of the salt ions. If any major differences existed between soft curd and hard curd milks in respect to salt balance we might hope to find

indications of it with these measurements. The concentration of lactose we have already seen appears to be quite constant for various milks so its effect may be cancelled out.

In table 10 are presented freezing point data obtained with the Hortvet cryoscope (12). The precision of the measurements is within a few thousandths of a degree. Each value is the result of three or more observations.

TABLE 10
Freezing point in relation to curd tension

CURD TENSION	FREEZING POINT
<i>gms.</i>	<i>°C.</i>
32	-.545
38	-.541
40	-.537
30	-.535
mean	-.540
102	-.535
102	-.547
125	-.555
mean	-.549

There is a mean difference of 0.009° C. in the freezing point of the two types of milk in favor of the hard curd milk which may indicate a somewhat higher salt concentration but a difference as small as this does not indicate a very different distribution of salt ions; moreover, the individual variations in the freezing point do not have a consistent direction.

The conductivity measurements were made with a Leeds and Northrup set-up (1927). The temperature employed was 25° C. and it was maintained with a precision of $\pm .05^{\circ}$ C. The results are presented in table 11.

TABLE 11
Conductivity in relation to curd character

KIND OF MILK	CONDUCTIVITY
Hard curd	<i>mhos.</i>
No. 3	42.7×10^{-4}
No. 38	44.8×10^{-4}
Soft curd	
No. 7	54.7×10^{-4}
No. 32	51.8×10^{-4}
No. 19	51.6×10^{-4}
No. 20	47.1×10^{-4}

Now and then exceptions have been found but in general the soft curd milks seem to have the greater conductivity.

Table 12 represents an attempt to narrow down the factors responsible for the difference in conductivity. The whey was obtained by centrifuging the whole milk at 40,000 r.p.m. for fifteen minutes. In column A we find a mean difference of 10 units in 48 and in column B, a difference of 6 units in 56, between the soft and hard curd milks. It thus appears that upon removing the suspensoid phase (casein, fat, calcium phosphates) that the conductivity of a soft curd and a hard curd milk tend to approach each other. It will also be noted that the *increase* in conductivity of the whey is greatest for the hard curd milks.

TABLE 12
Conductivity: whole milk versus corresponding whey

KIND OF MILK	A. MILK CONDUCTIVITY	B. WHEY CONDUCTIVITY
Hard curd	<i>mhos.</i>	<i>mhos.</i>
No. 3	42.7×10^{-4}	52.9×10^{-4}
No. 38	44.8×10^{-4}	53.6×10^{-4}
Mean	43.8	53.2
Soft curd		
No. 7	54.7×10^{-4}	59.8×10^{-4}
No. 10	52.7×10^{-4}	58.3×10^{-4}
Mean	53.7	59.1

The conductivity data seem to call for the following interpretation. The greater percentage of poor conducting substances (casein, calcium phosphate and fat) in the hard curd milk causes a lowered conductivity as compared to a soft curd milk. If the influence of the casein, phosphates and fat be cancelled out, there still remains a smaller difference in conductivity which may possibly be explained by the greater dilution of the constituents remaining in the whey of the soft curd milk.

B. Viscosity Measurements on Soft and Hard Curd Milks

It was thought that viscosity measurements might throw some additional light on the central problem. These measurements were carried out with Ostwald pipettes having an emptying time of about 30 seconds for 15 cc. of distilled water. A constant temperature of $25^{\circ} \text{C.} \pm .05^{\circ} \text{C.}$ was maintained by means of a well-insulated bath with an Aminco thermoregulating system. The results are expressed as relative viscosity, distilled water being the reference liquid. In table 13 are the data obtained.

It is evident that soft curd milks have a lower viscosity than the hard curd milks. From the data of table 13, a mean difference of approximately 15 per cent exists in relative viscosity between soft curd and hard curd milks.

Also in table 13 the viscosities of milks are compared with the viscosities of the corresponding wheys obtained from these milks by high speed centri-

TABLE 13
Relative viscosities of soft and hard curd milks

KIND OF MILK	RELATIVE VISCOSITY	RELATIVE VISCOSITY OF WHEY
Hard curd		
34-5	1.76	
22-5	1.68	
2-1	1.80	
Mixed hard curd	1.80	1.14
Soft curd		
11-1	1.53	
16-1	1.60	
28-5	1.57	
Mixed soft curd	1.55	1.13

fuging (40,000 r.p.m., 20 minutes). These data show that the differences in viscosity are associated with the suspensoid phase of the milk (calcium caseinate, calcium phosphates, fat).

These data are then in accord with the analytical data which showed that the concentration of casein, calcium, phosphorus, and often fat, was larger in hard curd milks than in soft; it is to be expected that the greater the percentage of these constituents the greater the viscosity.

C. *The pH in Relation to Curd Character*

Hydrogen ion concentration has long been known to be an important factor in the phenomena of rennet coagulation (13), even small changes in pH causing large changes in rennet coagulation.

To seek a relation between pH and curd tension, samples of normal milk were rendered alkaline with NaOH and other samples acid with HCl, the pH and corresponding curd tensions were then determined. For the pH

TABLE 14
Relation of pH to curd tension

DESCRIPTION OF MILK	INITIAL PH	PH AFTER CURD TEST	CURD TENSION
1 cc. N/1 NaOH 250 cc. milk	7.20	5.88	12
Untreated milk (control)	6.75	5.80	24.5
2 cc. N/1 HCl 240 cc. milk	6.35	5.54	44.5
4 cc. N/1 HCl 250 cc. milk	5.95	5.36	64.5

determinations a quinhydrone electrode with a precision of $\pm .03$ pH units was used. The results are given in table 14.

It will immediately be seen that the hydrogen ion concentration seems to have an overwhelming effect on the curd tension. The curd hardness increasing with increasing acidity and diminishing with increasing alkalinity.

Espe (4) found the same sort of effect working independently at Cornell. The next logical step was to determine the pH of various soft and hard curd milks in the hope that the same type of correlation might show itself. In table 15 are shown the results of this work.

TABLE 15
The pH as found with natural soft and hard curd milks

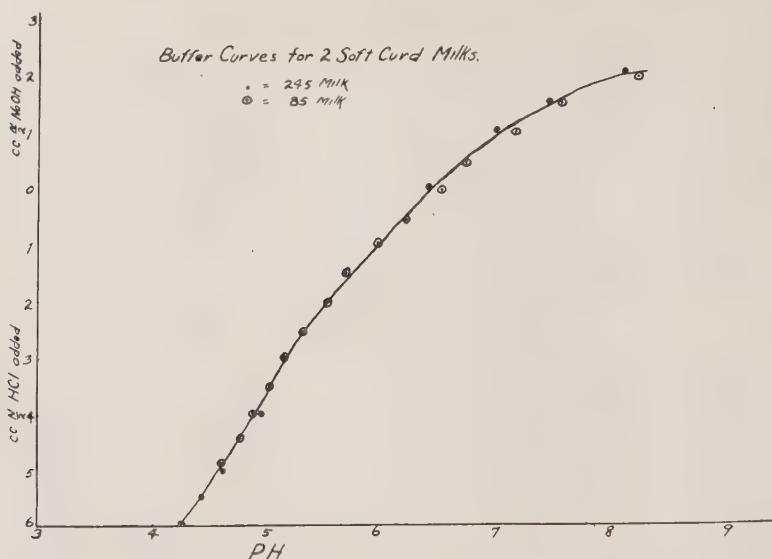
COW NO.	CURD TENSION	INITIAL pH	pH AFTER CURD TEST
4-4	155	6.55	5.70
37-5	145	6.55	5.60
31-4	138	6.55	5.70
5-4	100	6.50	5.60
32-4	85	6.48	5.70
28-4	60	6.61	5.70
8-5	55	6.60	5.70
24-5	50	6.50	5.78

The constancy of the pH in untreated milk is such as to eliminate this factor from the problem. Such variations as do exist show no correlation with curd tension. The sharp reduction in pH occurring during the test is owing to the presence of the calcium chloride. Calcium chloride is believed to react with the acid calcium phosphates of the milk liberating hydrochloric acid and tending to form tricalcium phosphate.

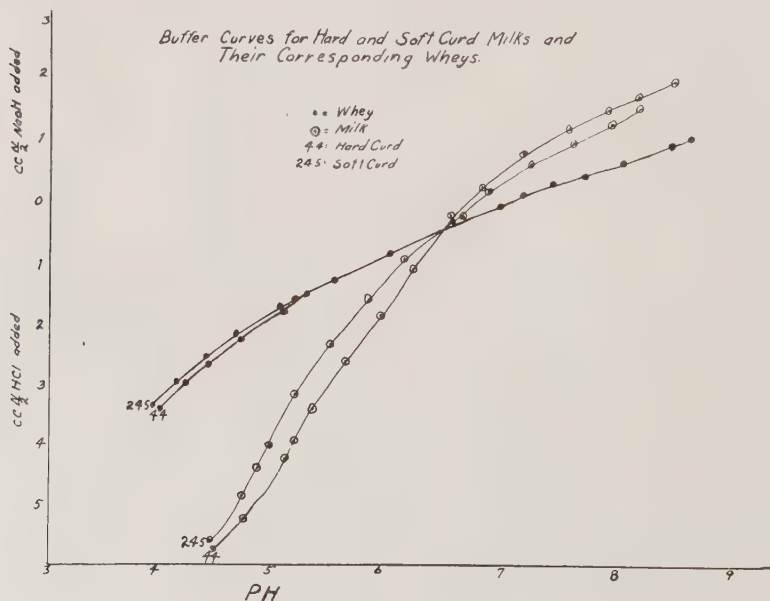
D. *Buffer Action in Relation to Curd Character*

Milk is a highly buffered system and many properties of milk have been associated with the buffer action of the milk. The chief buffer systems present in milk are the phosphate, citrate, carbonate, and protein (See "Fundamentals of Dairy Science," Chap. VI.) If buffer curves be constructed for a sufficient range of pH the different buffer systems will be brought into play. It was thought that by comparing such curves for hard and soft curd milks something could be learned of the fundamental properties of these milks. The attached curves were obtained by adding definite volumes, from a burette, of N/2 NaOH and N/2 HCl and determining the pH with a quinhydrone electrode the precision of which was $\pm .03$ pH units. Continuous rapid stirring was employed and the instrument was tested with a standard phthalate buffer solution at frequent intervals. Approximately five minutes were allowed for the milk to come to equilibrium before making a reading.

On the same graph were plotted the buffer curves for two soft curd milks (24-5, 8-5) and it was found that the two curves practically coincide



(see Graph I). The buffer curves for a hard curd milk (4-4) and a soft (24-5) were then plotted on one graph (Graph II) and the curves were seen to diverge. The indication was that the hard curd milk was more highly buffered over nearly the whole range, that is, it resisted change in pH more than the soft curd milk. This we would expect from the greater concentration of casein, Ca and P in hard curd milks.



Also on graph II are plotted the buffer curves for the wheys corresponding to 24-5 and 4-4 milks. These wheys were obtained in the customary manner by centrifuging the milks at high speed. It will be seen that the two curves coincide. This indicates clearly that the differences in buffer action were due to the suspensoid phase of the milk (calcium phosphates, fat, casein, etc.).

By plotting data for several hard curd milks, it was found that the buffer curves of the hard curded milks also vary to some extent among themselves. Accordingly, more extensive work will be necessary to confirm the apparently greater buffering action of hard curd milk, relative to soft curd milk.

SUMMARY OF PART II

1. The freezing point measurements sustain the concept that the salt balance of soft curd milk is not very different from that of hard curd milk.

2. Conductivity, viscosity, and buffer measurements tend to show that the suspensoid phase of the milk is chiefly responsible for the difference in properties between the soft and hard curd milk.

3. The pH seems to bear no relation to the curd tension in natural untreated individual milks.

PART III. PHYSICO-CHEMICAL EXPERIMENTS WITH SOFT CURD AND HARD CURD CASEINS

A. *Introduction*

It is conceivable that if there are structural differences in the casein from milk to milk the character of the milk curd would be influenced by such differences, as well as by the differences in concentration of the casein.

In Sutermeister's book (14) the impression is given that the composition of the casein for each species is probably constant, and also that it is constant from species to species. But the analyses on which this conclusion is based undoubtedly were made on mixed milk obtained from large numbers of animals especially in the case of cows, hence this conclusion does not rule out the possibility that the casein of any individual cow may have a composition characteristic for that cow.

In support of this conception is the work of Hugo Meyer (15). He found that in the case of human milk, each mother produces a milk which has a characteristic zone of flocculation at a constant temperature, the pH being varied in definite steps. He then prepared the caseins of each milk and found that these too when suspended in a salt solution had a characteristic flocculation zone which corresponded to the original milk in the sense that casein prepared from milk A always had the same flocculation zone and from milk B a different but characteristic zone.

In table 16 are shown some of his flocculation data.

TABLE 16
Flocculation zones for individual human milks

NAME	FLOCCULATION ZONE
Frau Elsa	pH 3.97-5.63
Frau Anna	pH 4.04-5.68
Frau Fanny	pH 3.88-5.29
Frau Lina	pH 3.80-4.72

F. Trendtel (16) apparently was able to confirm H. Meyer's work. He found that the isoelectric point of the caseins of individual human milks was found at hydrogen ion concentration varying from 2.19×10^{-5} to 7.95×10^{-5} and that the isoelectric point was constant for the casein derived from each woman's milk.

We attempted to throw light on this problem by making pure casein preparations from soft curd and hard curd milks and studying their properties.

B. *Preparation of the Casein from Hard and Soft Curd Milks*

Casein preparations were made from a hard and two soft curd milks employing essentially the method of Van Slyke and Baker (17) except that absolute ether was used as the last wash liquid. With this method the possibility of denaturing the casein is at a minimum. Casein No. 4-5 and casein No. 32-5 were the best preparations obtained. Their analyses are given in table 17.

TABLE 17
Percentage composition of casein preparations

CASEIN	PHOSPHORUS	ASH	PROTEIN	ALBUMIN	LACTOSE	MOISTURE
		<i>per cent</i>	<i>per cent</i>			<i>per cent</i>
32-5 (hard curd)	0.807	0.23	90.15	none	none	7.73
4-5 (soft curd)	0.756	0.12	91.05	none	none	8.05

C. *Tyrosine and Tryptophane Content in Relation to Curd Character*

Casein preparations No. 32 (hard curd), No. 38 (softer curd), No. 4 (softest curd) and "Difco" casein made from mixed cow's milk were analyzed for tyrosine and tryptophane by the method of Folin and Ciocalteu (18). (The standards used were made up from Eastman-l-tyrosine.) The data obtained are presented in table 18.

These data seem to show that the tryptophane content is reasonably constant in all the caseins but appreciable variations appear to exist in the tyro-

TABLE 18
Tyrosine and tryptophane content of individual cow caseins

COW NO.	CURD CHARACTER	TYROSINE	TRYPTOPHANE	DRY BASIS	
				Tyrosine	Tryptophane
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
32	Hard curd	7.60	1.62	8.22	1.75
38	Softer curd	7.90	1.88	8.65	2.05
4	Softest curd	6.85	1.66	7.45	1.81
*	From mixed milk	6.40	1.58	6.98	1.73

* Digestive Ferments Company.

sine content. However, the variations in tyrosine content do not seem to show a marked trend in relation to curd character.

More extended analyses on the amino acid content of these caseins would seem worthwhile.

D. *The Isoelectric Points of Soft and Hard Curd Casein*

The isoelectric point of a protein especially in the absence of salts may be expressed in terms of pH. It is the point at which the charge on the protein is zero. The solubility, viscosity, swelling, etc., are at a minimum.

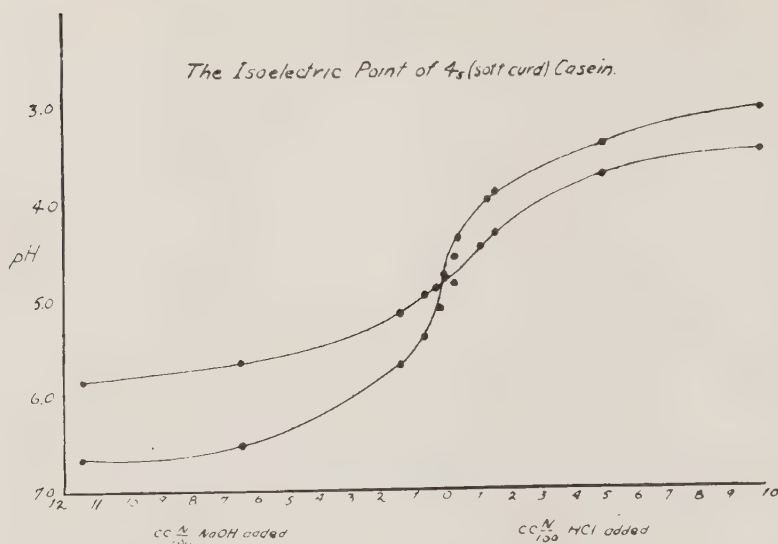
The determination was made in two ways: (1) by simply suspending the casein in CO₂ free distilled water and determining the pH with the quinhydrone electrode; (2) by making buffer curves for two concentrations of each casein, 0.50 per cent and 2.0 per cent. When these curves are plotted together they intersect in one and only one point—the isoelectric point. At all other points the difference in concentration of the casein causes a differing buffer action.

In table 19 are shown results obtained by method (1).

TABLE 19
Isoelectric points of soft and hard curd caseins

CASEIN	KIND OF CASEIN	TEMPERATURE	pH
<i>per cent</i>		° C.	
0.5	No. 32-5 (hard curd)	28	4.74
	No. 4-5 (soft curd)	28	4.74
1.0	No. 32-5 (hard curd)	34.5	4.77
	No. 4-5 (soft curd)	34	4.77

By method (2) an isoelectric point of 4.85 was obtained for No. 4-5 casein and one of 4.8 for No. 32-5 casein (see Graphs III and IV). The values obtained with CO₂ free water are in excellent agreement with those

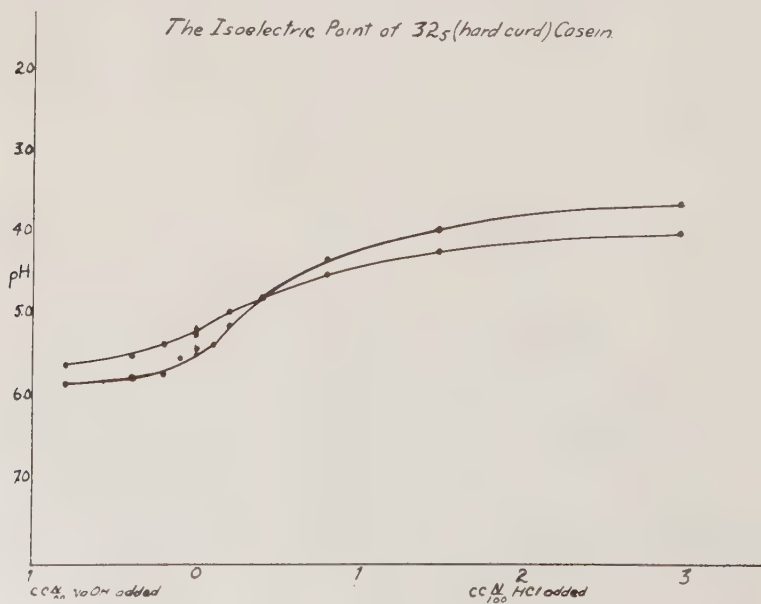


found by Hoffman and Gortner (19) who give 4.77 as the isoelectric pH for casein by this method.

Our data indicate that the soft and hard curd caseins had essentially the same isoelectric point.

E. Flocculation Zone of Hard and Soft Curd Casein

These casein preparations were suspended in a salt solution according to the procedure of S. Marui (20). Using the method of H. Meyer, which con-



sists of adding increasing amounts of N/20 hydrochloric acid, the flocculation zone of the two caseins was then determined. The results are presented in table 20.

TABLE 20
Flocculation zones of hard and soft curd caseins

N/20 HCl (cc.)	0	1	2	3	4	5	6	7	8	9	10
Kind of casein											
No. 32-5 (hard).....	-	-	-	+	+	++	+++	+++	++++	+	-
No. 4-5 (soft).....	±	±	-	+	+	+++	+++	+++	++	+	-
pH											
No. 32-5						4.65	4.35	4.20	3.90		
No. 4-5						4.78	4.50	4.20	3.93		

+ indicates flocculation.

- no flocculation.

These flocculation zones are seen to be essentially the same.

F. Viscosity of Solutions of Soft and Hard Curd Caseins

Equal amounts of casein 32-5 (hard) and casein 4-5 (soft) were dissolved in a saturated Ca (OH)₂ solution and viscosities determined. In another experiment dry ammonia gas was passed over a layer of each casein preparation in a long glass tube until the compound formed showed a pH of 6.3 to 6.4 in water solution. Then equal amounts of the compound were dissolved in water and viscosity determined. The results are presented in table 21.

TABLE 21
Viscosity of calcium and ammonium caseinates in relation to curd character. Temperature at 25° C.

KIND OF SALT	CASEIN NO.	VISCOSITY
		<i>sec.</i>
Calcium caseinate.....	32-5 (hard curd)	44.0, 44.0
Calcium caseinate.....	38-5 (soft curd)	46.4, 46.4
Ammonium caseinate.....	32-5 (hard curd)	46.3, 46.4
Ammonium caseinate.....	38-5 (soft curd)	46.4, 46.4

Table 21 shows no significant differences in viscosity. It was considered, however, that the concentration of casein may have been too small to reveal any differences. The concentration of casein used being about 0.5 per cent.

More concentrated solutions were then prepared using sodium borate which is a good solvent for casein. The results are plotted on Graph V and viscosities are tabulated in table 22.

TABLE 22
Viscosity of soft and hard curd casein in sodium borate solution. Temperature at 25° C.

CONCENTRATION OF CASEIN	VISCOSITY	
	Casein 4-5 (soft curd)	Casein No. 325 (hard curd)
<i>per cent</i>	<i>sec.</i>	<i>sec.</i>
0.0	30	30
	"	"
	"	"
1.0	35	36
	"	"
	"	"
3.0	48	49
	"	49
	"	48
4.0	56	60
	"	60
	"	61
5.0	74	81
	"	"
	"	"

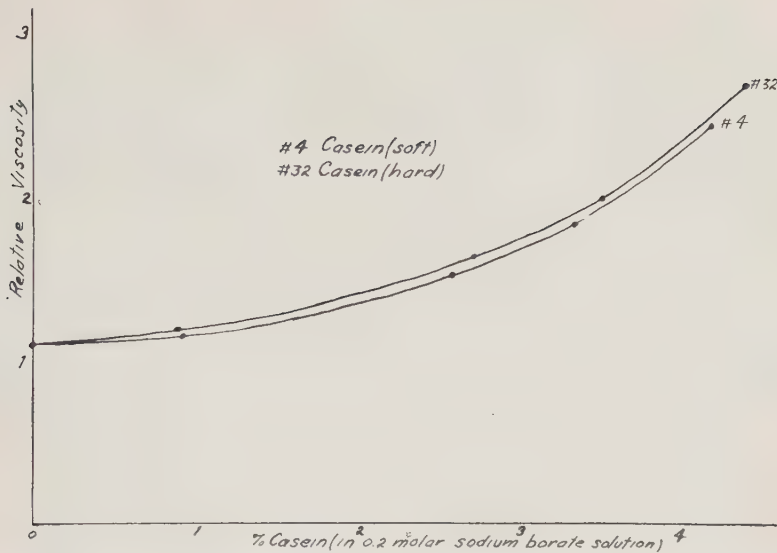
Note: The precise concentration of the casein was determined by nitrogen analyses on the solutions. It is these values that are plotted on the graph.

We do not believe that a difference of the magnitude seen on Graph V can be very significant.

The viscosity determinations, the isoelectric point determinations, and the flocculation zones do not serve to differentiate the caseins of the hard and soft curd milk with any sharpness. This however does not prove that the caseins are just alike. It may be that the sensitivity of these methods is not great enough for the purpose. It is conceivable that if a few of the constituent amino acids differed from one casein to another such a difference could not be detected by the determinations described above.

The recent work of Linderstrøm-Lang (21) and Svedberg and Carpenter (22) places the problem in a new light. Svedberg and Carpenter found by means of sedimentation analysis that casein prepared by the method of Hammersten consisted of 3 fractions of differing molecular weight. They, as is customary, doubtless made the casein from mixed milk obtained from several cows.

Linderstrøm-Lang during an extensive research found that casein prepared by the method of Van Slyke and Baker undoubtedly consisted of several fractions. He too doubtless worked with mixed milk from several cows. Linderstrøm-Lang (25) states "For the rest the possibility can not be ex-



cluded that milk from different cows and races of cows can vary in its composition, especially when casein is a mixture of several components, since a change of the proportions of mixing will not be in contrast with the apparently common experience that the chemical units in different kinds of organisms are very closely related to each other."

The direction in which to look for differences in the individual cow caseins thus is pointed out. It may be found to consist in differences in the fractions that go to make up each cow's casein. And these differences may somehow influence the curd character.

DISCUSSION

By correlating the results of quantitative analysis with measurements of viscosity, conductivity and buffer action on soft and hard curd milks, it seems to be established that the colloiddally dispersed phase differentiates a soft curd milk from a hard curd milk. That is, that the fat, casein, and calcium phosphates by reason of their concentration and manner of dispersion seem to control the curd character. (The qualitative make-up of the proteins may also contribute.)

The mechanism by which these constituents determine the difference between soft and hard curd milks may be as follows. The hard curd milk containing more casein should furnish more coagulating centers after peptic action and hence give a more rapid coagulation. In such event the micelles of casein would probably form a closely woven network from which the water would be squeezed out, resulting in a rather dense mass. The soft curd milk on the other hand having less casein would furnish fewer coagu-

lating centers; the micelles would extend farther before interlocking so that a relatively loose structure would result.

The colloidal calcium phosphates severed from the protecting action of the casein by the pepsin, in themselves, may furnish coagulating centers, hence the greater the concentration of calcium phosphates, the more rapid the coagulation, the more compact the micellar structure and the harder curdled the milk.

The fat may contribute to the determination of the curd character by interrupting the growth of the micellar threads of protein and so modify the structure. It will be apparent from this viewpoint that the manner in which the fat is dispersed would be as important as its concentration. Another possibility is that the amount of protein adsorbed by the fat varies considerably from individual milk to milk and that this influences the micellar structure which results upon peptic coagulation.

FINAL SUMMARY

1. It is established that a high concentration of casein is associated with hard curd milk and a low concentration with soft curd milk.
2. It is clearly indicated that the concentration and manner of dispersion of the fat are important in influencing the curd character.
3. A higher concentration of calcium and phosphorus is found in hard curd milk than in soft.
4. It is demonstrated that the rôle of the whey constituents in determining curd character must be a small one.
5. By correlating the results of quantitative analysis with measurements of viscosity, conductivity and buffer action, it seems to be established that the suspensoid phase differentiates a soft curd milk from a hard curd milk, that is, that the fat, casein, and calcium phosphates by means of their concentration and manner of dispersion seem to control the curd character.
6. Attempts to find decisive differences in composition between caseins from soft and hard curd milks were not successful but the possibility that such differences may exist is not wholly eliminated.

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